Medical Science

25(111), May, 2021

Effect of argan oil (*Argania spinosa*) on hypercholesterolemic male rats

Jamilah M Hashemi✉, **Salma A Alahmari**

To Cite:
Hashemi JM, Alahmari SA. Effect of argan oil (*Argania spinosa*) on hypercholesterolemic male rats. *Medical Science*, 2021, 25(111), 1150-1158

Author Affiliation:
Food and Nutrition Department, Faculty of Human Sciences and Design, King Abdul-Aziz University, Jeddah, Kingdom of Saudi Arabia

✉**Corresponding author**
Food and Nutrition Department, Faculty of Human Sciences and Design, King Abdul-Aziz University, Jeddah,
Kingdom of Saudi Arabia
Email: ghashemi@kau.edu.sa

Peer-Review History
Received: 02 April 2021
Reviewed & Revised: 04/April/2021 to 08/May/2021
Accepted: 09 May 2021
Published: May 2021

Peer-review Method
External peer-review was done through double-blind method.

ABSTRACT

Hypercholesterolemia is a metabolic condition that related to the occurrence of atherosclerosis and cardiovascular diseases. This research aimed to demonstrate the efficacy of argan oil in improving hypercholesterolemia in male rats. Forty male rats were randomly distributed into five groups (8 rats in each group); control; rats fed on normal rodent diet. The four remaining groups; rats were fed on a high-fat diet (HFD) for eight weeks to produce hypercholesterolemia. Then, the hypercholesterolemic rats were classified as follows: HFD, HFD + argan oil (5 ml/kg), HFD + statin (40 mg/kg), as a reference drug, and HED + argan oil + statin. After 4 weeks, blood samples were taken for biochemical examination. A liver histological analysis was also carried out. The results showed that treatment of hypercholesterolemic rats with argan oil, statin and their mixture significantly improved biological evaluation, reduced the levels of total cholesterol (TC), triglyceride (TG), low-density lipoproteins cholesterol (LDL-C), and atherogenic index (AI), concurrent with a significant increase in the level of high-density lipoproteins (HDL-C) versus HFD group. Furthermore, there was a significant decline in liver enzymes level and a significant decrease in oxidative stress; malondialdehyde (MDA) level versus HFD group. The group which were fed on HFD resulted in accumulation of lipid droplets of various size in liver tissue compared to control group. Argan oil administration showed protection against lipid deposition similar to statin medication. Better protection result was observed in the group which received a mixture of statin and argan oil.

Keywords: Hypercholesterolemia, argan oil, statin, lipid profile, oxidative stress, malondialdehyde.

1. INTRODUCTION

Hypercholesterolemia (HC) is characterized as high plasma cholesterol levels accompanied by normal plasma triglycerides and a rise in low-density lipoprotein (LDL) levels (Hervas and Ascaso, 2019). In Saudi Arabia, Basulaiman *et al*., (2014) assessed the hypercholesterolemia prevalence in addition to its related causes in Saudis aged 15 years or older. Hypercholesterolemia affected 8.5 % of Saudis. Another 19.6% had hypercholesterolemia on the verge of being hypercholesterolemic. Hypercholesterolemia is a major risk factors for occurrence of cardiovascular

diseases (CVDs) and as such many of the strategies to combat CVD have been founded when dealing with high levels of plasma cholesterol (Cannon *et al.,* 2015). More than a million Saudis suffer from hypercholesterolemia, with 700,000 unaware of their disease, which can be managed by early warning campaigns, lifestyle changes, and medication (Basulaiman *et al*., 2014). In Saudi Arabia, cardiovascular diseases (CVDs) are main reason of death. A total of 2047 people were enrolled in the PURE-Saudi sample. Overall, 69.4 %were physically inactive, 49.6 percent were obese, 34.4 % had an unhealthy diet, and 32.1 % had dyslipidemia (Alhabib *et al*., 2020).

Treatment options are limited among patients with hyperlipidemia; statins are the first line of treatment (Varbo *et al.,* 2014). Statins are widely used in the evidence-based as lowering of cardiovascular disease (CVDs) risk. Nonetheless, there has been some concern about the long-term consequences of statin use. Almost all statin medications have musculoskeletal side effects and are linked to an increased risk of diabetes mellitus. It has the potential to affect the kidneys and cause hypothyroidism. The risk tends to be greater between those taking higher doses of the statins. The argan oil obtained from argan tree (*Argania Spinosa L. Skeels*) seeds (Elabbassi *et al*., 2014). The high content of unsaturated fatty acids (UFA), especially oleic and linoleic acids, as well as antioxidants like tocopherols and phytosterols, contribute to argan oil's nutritional and therapeutic benefits (Elabbassi *et al*., 2014; Rueda *et al.,* 2017; Belcadi *et al.,* 2018).

The aim of this studywas to show that after four weeks of treatment, argan oil can improve the lipid profile of hypercholesterolemic male rats compared to statin, as reference drug.

2. MATERIAL AND METHODS

Diet, chemicals, drugs and kits

Baghafar Company for Pharmaceutical and Chemical, Jeddah, Saudi Arabia, provided the rats' chow pellets (normal diet) constituents. Pure Ghee and argan oil were purchased from the local market from Jeddah, KSA. Acros Organics Company provided cholesterol and bile salt. Statin (Astatin coated tablets, 40 mg) was obtained from pharmacy in Jeddah, SA. All diagnostic kits for estimating the lipid profiles, liver function enzymes, as well as ELISA kit for measuring lipid peroxidation were provided from My BioSource Company (*via* Mansour Scientific Foundation for Research and Development Company (MSFRDC)).

Experimental animals

Forty male Wistar Albino rats (130-170g) were offered from King Fahad Medical Research Center (KFMRC), KAU, Jeddah, Saudi Arabia. Rats were housed in a controlled laboratory environment. The rats were housed in well-aerated standard plastic cages with four rats per cage. These rats had unlimited access to water and were fed on a regular diet (laboratory chow) (Haimeur *et al.,* 2019).

Induction of hypercholesterolemia

For eight weeks, rats were fed a high fat diet (HFD) containing cholesterol powder (2%), pure ghee (20%), and bile salt (0.5%) to induce hypercholesterolemia (Zheng *et al.,* 2015; Pang *et al*., 2002). Following this time, blood samples were taken to determine total cholesterol levels (TC). Hypercholesterolemic rats were described as those with a blood cholesterol level of 300 mg/dl or higher (Kalsoom & Jafari, 2011). This experimental was started at November 2019 and continued for 13 weeks as follow; one week for adaptation, eight weeks for induction of hypercholesterolemia, then four weeks for treatment.

Experimental grouping

Forty rats were randomly assigned to five groups as follows: Control negative; rats were fed normal diet for 13 weeks, HFD group; rats were fed on HFD, HFD +argan oil group; rats were fed on HFD + argan oil (5 ml/kg/day) orally according to Berrada *et al*., (2000), HFD + statin group; rats were fed on HFD + statin (as reference drug) at a dosage of 40 mg/kg dissolved in distilled water (Musorowegomo, 2016), and HFD + statin + argan oil; rats were fed on HFD and given orally statin and argan oil. The rats were sacrificed at the end of the 13thweek of the experiment; blood samples plus the organ were obtained for biochemical and histopathological examination.

Biological evaluation

During experimental period, all rats were weighed at regular intervals (every week) using an electronic scale balance (AE ADAM Equipment, Inc. UK). The biological values for different groups were evaluated by body weight gain percent (BWG %) determination (Hijazi *et al.,* 2017). Feed intake (FI) weighed every day and feed efficiency ratio (FER) was calculated (Al Hamedan *et*

al., 2010). The liver and heart were carefully dissected, rinsed in saline and absolute organs weights were weighed using an electronic scale balance (AE ADAM Equipment, Inc. UK). The relative organs weights (ROW) were calculated (Tan *et al.*, 2018).

Biochemical analysis

Blood samples were centrifuged at 3000 rpm for 10 minutes (Hermle LaborTechnik GmbH - Z 200 A Universal Compact Centrifuge, Wehingen, Germany). Serum samples were used for the determination of lipid profile parameters (TC, TG, HDL-C, and LDL-C) according to manufacturer instructions. While VLDL-C and atherogenic index (AI) were calculated. Liver function enzymes (AST, ALT, and ALP) activities were determined according to manufacturer instructions. Malondialdehyde (MDA) was determined by Quantitative Sandwich ELISA kit for rats according to manufacturer instructions.

Histopathological examination

The liver samples in each group were put in a 10% neutral buffered formalin solution. The fixed specimens were then trimmed, washed, and dehydrated in increasing concentrations of alcohol, then cleared in xylene and stained with Hematoxylin and Eosin (H&E) (Grizzle *et al*., 2008). A light microscope was used to examine them (Olympus BX51-USA).

Statistical analysis

The data were presented as mean ± standard deviation (SD) and were analyzed by the Statistical Package for Social Sciences (SPSS) for Windows, version 25 (IBM SPSS, Corp., Armonk, N.Y., USA). Comparisons between different groups were made by One-Way analysis of variance (ANOVA) followed by the least significant difference test (LSD). The results were considered statistically significant if p-values were ≤ 0.05.

3. RESULTS AND DISCUSSION

Effect of argan oil and/or statin on biological evaluation in hypercholesterolemic rats

There was a significant decrease in BWG percent ($p< 0.001$) in argan oil-treated HFD (5 ml/kg) rats compared to the HFD group. Similarly, oral statin (40 mg/kg) administration to HFD rats resulted in a significant reduction ($p<0.001$) in BWG percent as compared to the HFD group. HFD rats received an oral mixture of argan oil (5 ml/kg) and statin (40 mg/kg) that resulted in a significant reduction in body weight. Meanwhile, there was an insignificant between hypercholesterolemic groups treated with either argan oil alone or statin alone. However, when comparing HFD rats treated with both argan oil and statin at the same time to HFD rats treated with either argan oil or statin alone ($p<0.001$), there were major differences (Table 1).

Table 1 Effect of argan oil and/or statin on biological evaluation in hypercholesterolemic rats

Experimental groups	Initial body weight(g)	Final body weight (g)	Weight gain percent
Control	145.67±11.76	324.00 ± 17.52	122.43±14.91
HFD	151.50 ± 11.11	363.38 ± 19.35 $^{a\wedge}$	139.86± 14.90 $^{a\wedge}$
HFD +argan oil	155.38±12.14	327.38 ± 24.94 $^{b\#}$	110.70±14.55 $^{b\wedge}$
HFD + statin	156.87±12.18	339.0 ± 26.02 ^{b*}	116.10±14.15 $^{b\wedge}$
HFD +argan oil + statin	152.25±9.89	279.63 ± 15.9 $^{b\wedge,c\wedge,d\wedge}$	83.67±14.73 $^{b\wedge,c\wedge,d\wedge}$

Data were represented as mean ± SD.

^{a}: significance versus control group; ^{b}: significance versus HFD group; ^{c}: significance versus argan oil; ^{d}: significance versus statin. ($^{*}p \le 0.05$, $^{\#}p \le 0.01$ and $^{\wedge}p \le 0.001$).

The FI of rats fed HFD was found to be significantly lower than control group in the current study, as shown in (Table 2). These findings corroborated previous findings (Haimeur *et al*., 2019). The reduction in FI in HFD group, likely as a result of long-term HFD administration, resulted in an inhibitory effect on basal plasma ghrelin levels in male rats (Gomez *et al*., 2003). The present study showed that rats fed on HFD had a significant increase in FER versus the control group. In argan oil (5 ml/kg) or statin (40 mg/kg) -treated HFD rats, there was a significant decrease in FER ($p \le 0.001$) compared to the HFD group. A mixture of argan oil + statin to HFD rats resulted in a significant decrease in FER versus the HFD group. These results could be explained by that argan oil administrations improve glucose, insulin and lipid profile, leptin and leptin resistance that are associated with obesity. Leptin supposedly causes lower energy intake; however, elevated leptin levels in obese rats did not suppress appetite due to leptin resistance (Chai *et al*., 2014).

Table 2 Effect of argan oil and/or statin on FI and FER in hypercholesterolemic rats

Experimental groups	FI (g/rat/day)	FER
Control	23.78 ± 0.95	0.067 ± 0.0057
HFD	20.91±1.65 [a^]	0.092 ± 0.0111 [a^]
HFD + Argan oil	20.61±1.09	0.076 ± 0.006 [b^]
HFD + Statin	19.86 ±1.78	0.082 ± 0.003 [b^]
HFD + Argan oil + Statin	16.20 ±1.33 [b^,c^,d^]	0.070 ± 0.005 [b^,c*,d#]

Data were represented as mean ± SD.

[a]: significance versus control group; [b]: significance versus HFD group; [c]: significance versus argan oil; [d]: significance versus statin. ([*]p ≤ 0.05, [#]p ≤ 0.01 and [^]p ≤ 0.001).

In the current research, the HFD group revealed significant increases in liver weight, relative liver weight, heart weight, and relative heart weight compared to the control group. The obtained results could be due to an increase in adipose tissue deposited in these organs. In argan oil-treated HFD (5 ml/kg) rats, there was a significant decline in liver weights versus the HFD group. Treatment of HFD rats with a statin (40 mg/kg) significantly decreased the relative liver weight compared to argan oil treated rats and the HFD group, as well as heart weight compared with the HFD group as depicted in (Table 3). This effect could be due to improvement of lipid profile which is confirmed in the biochemical results. Besides, a mixture of argan oil and statin was the most effective in reducing liver weight, relative liver weight, and heart weight compared with HFD and HFD either treated with argan oil alone or statin alone. The decrease in relative adipose tissue weights led to decreased different organ weights. In this respect, it was reported that argan oil treatment reduced relative adipose tissue weight in rats fed a normal fed diet and HFD, which led to a decrease in relative organ weights (Sour *et al.*, 2015).

Table 3 Effect of argan oil and/or statin on liver and heart weight and their relative weight in hypercholesterolemic rats

Experimental groups	Liver weight (g)	Relative liver Weight (%)	Heart weight (g)	Relative heart weight (%)
Control	8.03±0.48	2.48±0.14	0.80±0.037	0.246±0.021
HFD	9.87 ± 0.42 [a^]	2.72±0.21 [a^]	1.05±0.077 [a^]	0.289± 0.019 [a^]
HFD + Argan oil	8.92±0.46 [b^]	2.72±0.23	0.99±0.079	0.302±0.018
HFD + Statin	8.53±0.39 [b^]	2.52±0.19 [b*, c*]	0.94±0.054 [b#]	0.278±0.038
HFD+ Argan oil + Statin	8.11±0.29 [b^,c^,d*]	2.91± 0.09 [b*,c*,d^]	0.82±0.076 [b^,c^,d#]	0.293 ±0.017

Data were represented as mean ± SD.

[a]: significance versus control group; [b]: significance versus HFD group; [c]: significance versus argan oil; [d]: significance versus statin. ([*]p ≤ 0.05, [#]p ≤ 0.01 and [^]p ≤ 0.001).

Effect of argan oil and/or statin on lipid profile parameters and atherogenic index in hypercholesterolemic rats

In the current analysis, the lipid profile of rats fed the HFD revealed an elevation in TG, LDL-C, TC, and VLDL-C values, as well as a decline in HDL-C. Tables (4 and 5) shows that the HFD group had a substantial elevation in serum TC, TG, LDL-C, VLDL-c and AI, along with a decline in HDL-C level, indicating successful induction of hypercholesterolemia and hyperlipidemia in rats, which causes atherosclerosis. LDL-C is known as bad cholesterol because it causes cholesterol to build up in the arteries (Mouhib *et al.*, 2017). Although HDL-C is known as "healthy cholesterol," it protects against cardiovascular disease by acting as an emergency carrier of cholesterol from peripheral cells to the liver (Kesh *et al.*, 2016). Previous researches revealed the beneficial actions of argan oil on blood lipid profiles in humans and animals (Berrougui *et al.*, 2006; Sour *et al.*, 2012; Sour *et al.*, 2015 & Haimeur *et al.*, 2019). Argan's hypolipidemic and hypocholesterolemic properties are due to its phenolic fraction, which inhibits LDL-C oxidation, elevated cholesterol efflux from T-helper precursor-1 macrophages, and improves reverse TC transport by improving HDL-C lipid bilayer fluidity (Berrougui *et al.*, 2006).

In this research, rats that were feeding HFD revealed a significant elevation in AI in comparison to control group. In this respect, it was reported that HFD is effective to produce hyperlipidemia and hypercholesterolemia in rat's model as reported by (Otunola *et al.*, 2010); (Noeman *et al.*, 2011) and (Miller *et al.*, 2011). This could explain *via* HFD increases chylomicrons numbers in the small intestine that when enter into general circulation led to free fatty acids (FFAs) formation that is taken by the liver. These hepatic

FFAs either esterified to form TG or enter mitochondria for β oxidation. These TGs are either accumulated in hepatocytes as small fat droplets or form VLDL-C that converts into LDL-C (Kesh *et al*., 2016).

Table 4 Effect of argan oil and/or statin on total cholesterol (TC), triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) in hypercholesterolemic rats

Experimental groups	TC (mg/dl)	TG(mg/dl)	HDL-C (mg/dl)
Control	90.50 ± 8.99	75.25 ± 4.53	47.13 ± 2.90
HFD	204.75 ± 17.64 $^{a\wedge}$	345.38 ± 14.93 $^{a\wedge}$	26.25 ± 4.65 $^{a\wedge}$
HFD + Argan oil	95.25 ± 5.92 $^{b\wedge}$	84.00 ± 7.93 $^{b\wedge}$	39.00 ± 3.21 $^{b\wedge}$
HFD + Statin	86.50 ± 7.21 $^{b\wedge}$	80.88 ± 7.77 $^{b\wedge}$	42.25 ± 4.39 $^{b\wedge}$
HFD+ Argan oil + Statin	74.00 ± 6.97 $^{b\wedge,c\wedge,d*}$	71.50 ± 3.59 $^{b\wedge,c\#,d*}$	46.71 ± 5.31 $^{b\wedge,c\#,d*}$

Data were represented as mean ± SD.

^{a}: significance versus control group; ^{b}: significance versus HFD group; ^{c}: significance versus argan oil; ^{d}: significance versus statin. ($^{*}p \leq 0.05$, $^{\#}p \leq 0.01$ and $\wedge p \leq 0.001$).

Table 5 Effect of argan oil and/or statin on low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C) and Atherogenic Index (AI) in hypercholesterolemic rats

Experimental groups	LDL-C(mg/dl)	VLDL-C(mg/dl)	AI
Control	73.25 ± 7.55	15.05 ± 0.86	0.204 ± 0.030
HFD	227.75 ± 26.29 $^{a\wedge}$	69.09 ± 1.42 $^{a\wedge}$	1.119 ± 0.076 $^{a\wedge}$
HFD + Argan oil	91.50 ± 10.69 $^{b\wedge}$	16.80±1.70 $^{b\wedge}$	0.333 ± 0.052 $^{b\wedge}$
HFD + Statin	88.00 ± 8.21 $^{b\wedge}$	16.18 ±1.37 $^{b\wedge}$	0.282 ± 0.023 $^{b\wedge}$
HFD+ Argan oil + Statin	68.14 ± 9.23 $^{b\wedge,c\wedge,d*}$	14.30 ± 2.01 $^{b\wedge,c\wedge,d*}$	0.185 ± 0.004 $^{b\wedge,c\wedge,d\wedge}$

Data were represented as mean ± SD.

^{a}: significance versus control group; ^{b}: significance versus HFD group; ^{c}: significance versus argan oil; $^{d:}$ significance versus statin. ($^{*}p \leq 0.05$, $^{\#}p \leq 0.01$ and $\wedge p \leq 0.001$).

Liver function enzymes

The results of this study revealed that levels of ALT, AST, and ALP in the serum of the HFD group were significantly elevated than control group. Many previous studies have identified an increase in liver enzyme activities in the HFD population, corroborating these findings (Abbas and Sakr, 2013; Sour *et al*., 2012). In this research, daily administration of argan oil or statin or their mixture lowered serum values of ALP, AST and ALT versus HFD group as depicted in (Table 6). Thus, indicating ameliorating hepatocyte damage in these groups. The improvement effect of argan oil could be due to its anti-inflammatory and antioxidant actions which protecting liver cells against membranous lipid peroxidation and consequent enzyme leakage (El Kamouni *et al*., 2017 & Bakour *et al*., 2018).

Table 6 Effect of argan oil and/or statin on aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) in hypercholesterolemic rats

Experimental groups	ALT(U/L)	AST(U/L)	ALP (U/L)
Control	15.24 ± 2.34	19.23 ± 3.23	44.63 ± 3.78
HFD	110.88 ± 9.55 $^{a\wedge}$	148.13 ± 10.49 $^{a\wedge}$	181.50 ± 5.68 $^{a\wedge}$
HFD + Argan oil	18.99 ±1.87 $^{b\wedge}$	24.01 ± 3.05 $^{b\wedge}$	51.75 ± 7.44 $^{b\wedge}$
HFD + Statin	21.76 ± 2.71 $^{b\wedge}$	28.49 ± 6.21 $^{b\wedge}$	55.75 ± 6.45 $^{b\wedge}$
HFD+ Argan oil + Statin	13.23 ± 2.19$^{b\wedge,c*,d\#}$	17.69 ± 2.29 $^{b\wedge,c*,d\#}$	45.29 ± 3.99$^{b\wedge,c*,d\#}$

Data were represented as mean ± SD.

^{a}: significance versus control group; ^{b}: significance versus HFD group; ^{c}: significance versus argan oil; ^{d}: significance versus statin ($^{*}p \leq 0.05$, $^{\#}p \leq 0.01$and $\wedge p \leq 0.001$).

Oxidative stress biomarker

In this study, after 13 weeks, rats that were feeding HFD showed a significant elevation in oxidative stress as evidence by a significant elevation in MDA serum level versus control group. Increased hepatic and heart oxidative stress is associated with a decrease in antioxidant enzyme activities, which is followed by an increase in MDA levels in most tissues (Figure 1) (Noeman *et al.*, 2011). Hypercholesterolemia, irregular metabolism, metabolites originating in adipose tissue, and/or excessive inflammatory and proinflammatory cytokine formations can all contribute to raise values of oxidant markers in obese rats (Ozata *et al.*, 2002 & Leopold and Loscalzo, 2008).

The present result indicated that ingestion of argan oil, statin, and their mixture in HFD rats leads to a significant decline in oxidative stress as evidence by a decline in MDA serum level in comparison to HFD group. Argan oil was more effective than a statin, and a combination of argan oil and statin was the most effective as compared to either argan oil alone or statin alone, suggesting a decrease in lipid peroxidation and cellular injury that protects the liver from HFD-induced oxidative harm (Haimeur *et al.*, 2019).

The argan oil protective action may be speculatively attributed to the antioxidant properties of its tocopherols and polyphenols which decreased oxidative stress (Drissi *et al.*, 2004). Moreover, many clinical trials revealed the benefits of argan oil on lipid serum profile and oxidative stress. This could attribute to phenolic, tocopherol, and saponin constituents of argan oil which provide powerful antioxidant actions (Cherki *et al.*, 2005).

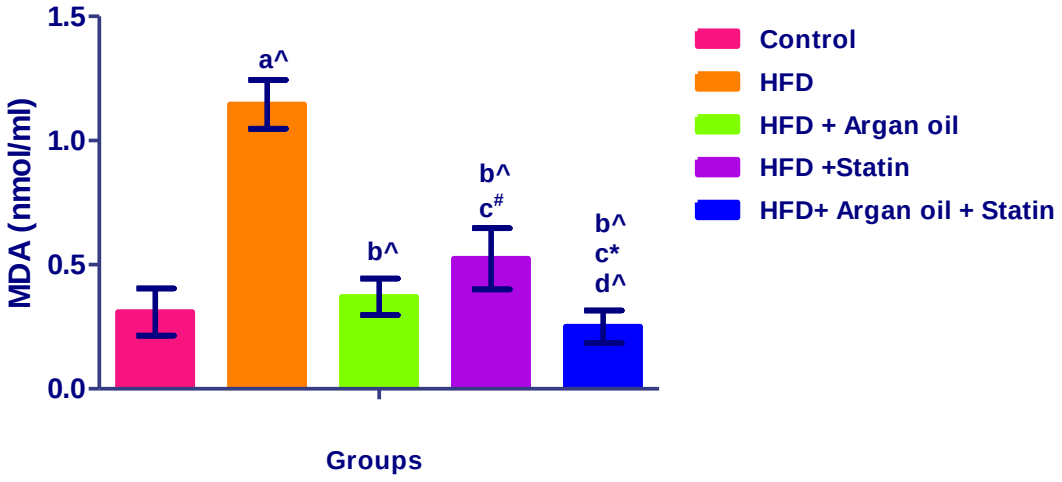

Figure 1 Effect of argan oil and/or statin on malondialdehyde (MDA) in hypercholesterolemic rats

Data were represented as mean ± SD. [a]: significance versus control group; [b]: significance versus HFD group; [c]: significance versus argan oil; [d]: significance versus statin. ($^{*}p \leq 0.05$, $^{\#}p \leq 0.01$ and $^{\wedge}p \leq 0.001$).

Histopathological examination

Histopathological examination of the liver tissue demonstrated that HFD induced deposition of large unstained vacuoles (lipids) within numerous hepatocytes located near the central vein region. Focal cell necrosis was also observed. Liver tissue at PA showed also marked deposition of rounded lipid droplets of various sizes within hepatocytes versus the control group (Figures 2, 3 A and B, for control and HFD group respectively). These results are in line with Altunkaynak and Ozbek (2009).

In HFD and argan oil or statin, most hepatocytes looked normal with active nuclei, and a marked absence of lipid deposition, except for few cells which showed small fat droplets that were associated with slight sinusoid dilation. While PA region showed an absence of any fat deposition in hepatocytes (Figures 2, 3 C and D for HFD+ argan oil and HFD+ statin, respectively). These results agree with Zhang *et al.*, (2020). In HFD treated with a mixture of argan oil and statin, the liver section at both CV and the PA regions showed normal hepatocytes structure with absence of any lipid deposition (Figures 2, 3 E.). Thus, it appeared that this therapy was the most superior as hypolipidemic agents. No literature is available regarding using argan oil as a natural adjuvant with a statin and this could be with an advantage in the future to avoid statin side effects.

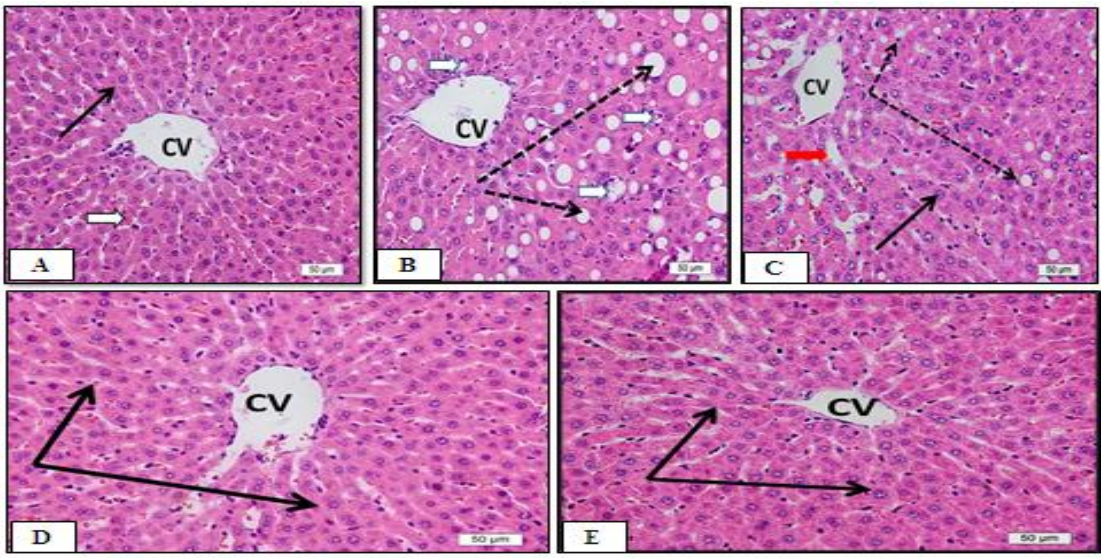

Figure 2 A Photomicrograph of a liver section at central vein (CV) region (H & E stain X 20, scale bar 50 μm). Control rats showing normal central vein (CV) surrounded by regular hepatocytes cell cords (arrow) and blood sinusoids (Fig. A). HFD rats showing deposition of large unstained vacuoles (lipids) within numerous hepatocytes (dotted arrows). Focal cell necrosis also observed (white arrows) (Fig. B). HFD + Argan oil rats showing most hepatocytes looked normal with active nuclei (black arrows) with a marked absence of lipid deposition, except for few cells that showed tiny fat droplets (dotted arrows). Slight sinusoid dilation could be observed (red arrow) (Fig. C). HFD + Statin rats showing hepatocytes looked healthy similar to control with the absence of any lipid droplets within the cells (black arrows) (Fig. D). HFD +Argan oil + Statin rats showing marked preservation of normal hepatocytes (black arrows) structure with the absence of any lipid deposition (Fig. E).

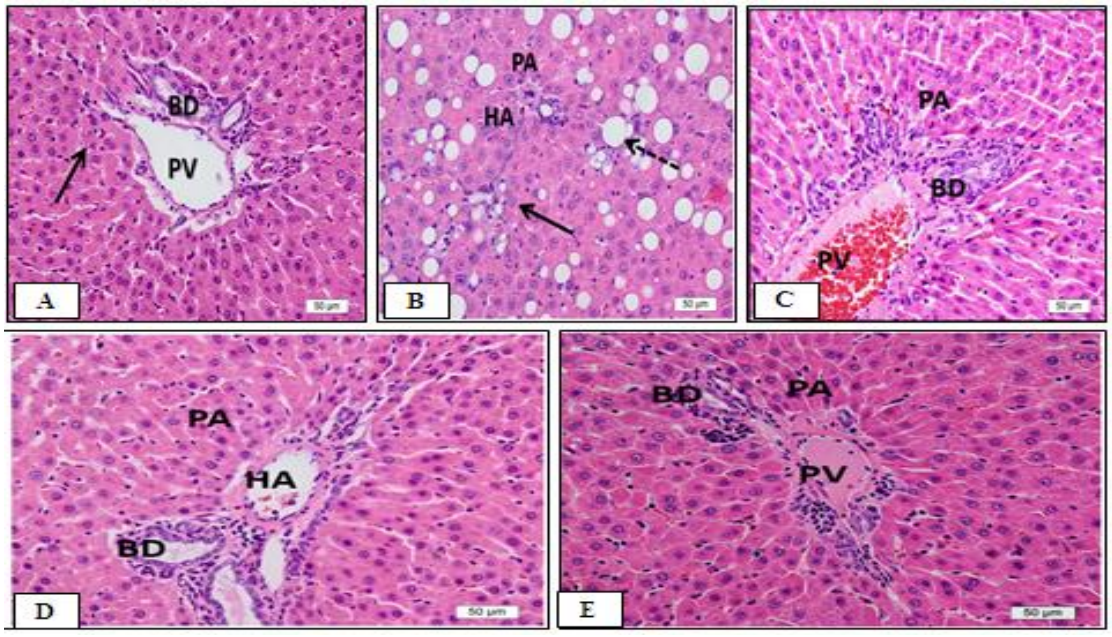

Figure 3 A Photomicrograph of a liver section at portal area region (P.A.) (H&E stain X 20, scale bar 50 μm). Control rats showing normal portal vein (P.V.) and bile ducts (B.D) surrounded by a tiny amount of connective tissue (arrow (Fig. A). HFD rats showing marked deposition of rounded lipid droplets of various sizes (dotted arrows) within hepatocytes and branch of the hepatic artery (Fig. B). HFD + Argan oil rats showing the absence of any fat deposition in hepatocytes, portal vein (P.V.), congestion and few inflammatory cells around the bile duct (B.D) (Fig. C). HFD + Statin rats are showing the absence of fat deposition in hepatocytes, similar observation to HFD+ Argan oil, normal portal vein (P.V.) and bile duct (B.D.), except slightly inflammatory cells around the portal area (Fig. D). HFD + Argan oil + Statin rats showing the absence of fat deposition in hepatocytes, with the normal portal vein (P.V.) and bile duct (B.D) (Fig. E).

4. CONCLUSION

The results of this research confirmed that daily intake of argan oil (5 ml/kg BW) for 4 weeks exerted hypolipidemic and hepatoprotective effects as statin, plus it has antioxidant activity in hypercholesterolemic rats. This was observed *via* improvement in biological evaluation, reduction of lipid profile parameters, liver enzymes and oxidative stress biomarker (MDA) level. Besides,

notable improvement on liver tissues compared to the HFD untreated group. The mixture of argan oil and statin was the most effective treatment compared with either argan oil or statin alone.

Ethical approval

This study protocol was approved by The Research Ethics Committee Unit, Faculty of Medicine - KAU (Reference No 751-19).

Acknowledgement

The authors gratefully acknowledge Prof. Dr. Soad Shaker for her sincere assistance. As well as the authors' appreciative acknowledgement to the Food and Nutrition Department – King Abdul Aziz University for their valuable assistance and care.

Authors Contributions

Prof. Dr. Jamilah Hashemi: made the research idea, study design, manuscript revision, and corresponding author. Salma Alahmari conducts the experimental model, collect samples, results discussion, and writing the manuscript.

Funding

This study has not received any external funding.

Conflict of interest

The authors declare that there are no conflicts of interests.

Data availability

All data associated with this study are present in the paper.

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